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INSECTICIDAL ACTIVITY OF DIFFERENT BOTANICALS (BITTERAPPLE, NEEM AND TOBACCO) TOWARDS *TRIBOLIUM CASTANEUM* (COLEOPTERA: TENEBRIONIDAE)

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Tribolium castaneum is the most destructive insect pest of stored products. The heavy infestation of this insect pest causes massive damage to cereals both qualitatively and quantitatively. Many botanicals have been used against *Tribolium castaneum* (Coleoptera: Tenebrionidae) as they have insecticidal properties. This experiment was conducted to evaluate mortality and repellency effect of Neem, Bitterapple and Tobacco against *Tribolium castaneum* on filter papers. Three different concentrations of extracts of each plant viz. 5.0%, 10%, 15% were taken after different time periods (24, 48, and 72 and 96 hrs.) from stock solution prepared. All concentrations of each botanical showed well effectiveness as repellent against *Tribolium castaneum* (Neem; 25.67%, Bitterapple; 26.56%, and Tobacco; 23.89%). Among these, the better result of repellency was observed in bitter apple. Mortality effect was maximum observed in Tobacco at 10% concentration after 24 and 48 hours' interval (70%.86%, 76.0%). Other results of Neem and Kortumba were also significant. Neem depicted more toxic results after Tobacco at 5% and 10% concentrations respectively. Moreover, Neem showed more repellent effect than toxicity. These results suggest that the plant extracts evaluated in this study may be useful in repellent and toxicant formulations against *T. castaneum*.

Keywords: ***Tribolium*** *CASTANEUM*, toxicant formulations, Insecticidal response

INTRODUCTION

Safe storage of grains and food products against insect damage is a serious concern (Haq *et al*., 2005). Stored grain infestation is a very serious problem as various life stages of insects cause economic damage and deteriorates the quality of food grains and food products. (Sarwar *et al*., 2015). It has been estimated that about 9% of the world's grain production is lost to post harvest insect and mite's infestations (Tooba *et al*., 2005) due to favorable climatic and storage conditions (Rahman *et al*., 2009). Traditional grain storage facilities may not offer protection, but promotion of the use of metal silos and resistant varieties for grain storage is an alternative approach to reduce losses (Tadele *et al*., 2011). The drying of the foods helps in reducing the moisture content to about 9-12% in the drier areas, thus, minimizing the activities of storage insect pests and pathogens (Okunade *et al*., 2001).

There are a number of stored grain insect pests that infest food grains in farmer stores and public warehouses and massively surge due to un-controlled environmental conditions and poor warehousing technology used (Sarwar *et al*., 2012). Primary stored grain insect pests include Lesser Grain Borer (*Rhyzopertha dominica*) (Fabricius), Granary, Rice and Maize Weevils (*Sitophilus* spp.), Angoumois Grain Moth (*Sitotroga cerealella*) (Oliver) Secondary Pests of Stored products are Flour Beetles (*Tribolium confusum* and *Tribolium castaneum*), Saw-toothed Grain Beetle (*Oryzaephilus surinamensis*) (Linnaeus), Flat Grain Beetle (*Cryptolestes ferugineus*) Steph, Warehouse Moth (*Ephestia elutella*) (Hubner), Indian Meal Moth (*Plodia interpunctella*) (Hubner) and Khapra beetle (*Trogoderma granarium*) Everts (Sarwar *et al.,* 2015). All stages (egg, larva, pupa, and adult) of each stored grain insect pest may be present simultaneously in infested stored products (Ali *et al*., 2011).

Rust-red flour beetle (*T. castaneum*) (Herbst) is frequently found on farms and it is a reddish brown beetle about 3 mm long. The final three segments of its antennae are greatly enlarged to form a club shape. Young adults are pale brown in color becoming darker with age. Females lay up to 1000 eggs loosely scattered throughout infested grain.

Cream-colored larvae with biting mouth parts and three pairs of legs hatch, and remain free from the grain, feeding on cereal dust and damaged grains. A generation takes about one month to complete under summer conditions, but longer in cold weather. The adult is winged and may fly and can live up to a year. (Sarwar *et al*., 2015)

Tribolium castaneum is a very destructive insect pest of food grains and stored grain products (Nadeem *et al.,* 2012). It lives in and feeds on wheat flour (Lu *et al.,* 2010). The Larvae of *Tribolium castaneum* destroy 12.5-14.60 % of weight of

individual seeds and approximately 88 grains are attacked by larvae (Wakil *et al.,* 2003). The entire life cycle of *Tribolium castaneum* lasts about 30-80 days while adult can live for 3 years into grain storage containers (Singh *et al.,* 2006).

It is necessary to conserve the stored food grains reserves so that the supply food remain continuous and the prices of food grains and derived products remain stable (Jahromi *et al.,* 2014). For this purpose, the occurrence of *Tribolium castaneum* is primarily controlled by fumigant insecticides (Klementz *et al.,* 2008). However, resistance has been developed due to their consistent and repetitive use against this pest (Kumar *et al.,* 2011). Though a huge progress in pest control has been achieved by chemical measures, its adverse effect on ecological system and human life has stressed the need to develop alternative methods for controlling the various arthropod pests (Risk *et al.,* 2001).

Under such conditions, the use of bioactive pesticides for protection of stored grains would be a safe alternative (Epidi *et al.,* 2009). The easiest way to use botanical insecticides against stored grain pest consist on drying the foliage and then mixing it with the grain. However, if fumigant effect is required, the essential oil is a better option (Juan *el al.,* 2016). The botanical insecticides have been used as powders, extracts, and essential oils for many years. These pesticides have shown contact, fumigant, antifeedant, and repellent activity against insects (Silva *et al*., 2003). Many plants like *Annona squamosa* (L.), *Lantana camara, Clerodendrun inerme, Cassia fistula, Azadirachta indica* and *Calotropis procera* are proved to be lethal to various stored grain pests and delay the developmental stages by interfering with their apolytic and molting processes (Deka *et al.,* 2005). These plants have shown insecticidal, antifeedant, repellant and growth regulating properties (Sankari *et al.,* 2007; Nirjara *et al.,* 2010). Studies of essential oils extracted from other plants that contain terpinen-4-ol, 1, 8-cineole or cymol as the main components, have shown insecticidal activity against insect pests of stored products (Tapondjou *et al*., 2005). The main secondary metabolite identified with insecticidal properties is the (*E*)-beta-bergamotene which is a sesquiterpene identified as a volatile compound released by plants in response to insect herbivory (Zhuang *et al*., 2012). Huang *et al*. (2002) indicated that safrole has insecticidal activity against *S. zeamais* and *Tribolium castaneum* (Herbst). Similarly leaf powders of *Annona squamosa* and *Balanites aegyptica* (L.) caused high mortality in *T. castaneum* and provided protection against seed damage (Ahmed *et al.,* 2009). So, botanicals are traditionally and widely used as stored grain protectants due to their easy accessibility and biodegradable nature (Dwivedi *et al.,* 2003). Production of repellents from plants is less expensive and easy as compared to synthetic chemicals (Shadia *et al.,* 2011). Many plant extracts are used in different forms such as *essential* extracts and powders and they are proved to be used as stored products repellents that are economically important (Mondal *et al.,* 2014).

There is a need to produce such environment friendly alternatives which have the potential to replace highly toxic chemicals. The present study is being performed to evaluate the biological activity of plant extracts against the *Tribolium castaneum.* The reason for selection of plants is that they are easily available in Punjab and these plants are also cheaper source of bio-chemicals against *Tribolium castaneum.* So, this project will be carried out to demonstrate the effect of botanicals with combination and alone against *Tribolium castaneum*. The outcomes of this study will be helpful for planning the grain protection in the storage.

MATERIALS AND METHODS

***Experimental Site*:** The research project was carried out in the Stored Grain Laboratory at Department of Entomology, College of Agriculture, Bahauddin Zakariya University Bahadur Sub-Campus Layyah. The material comprised of neem (*Azadirachta indica*), Kortuma (*Citrullus colocynthis*), Tobacco (*Nicotiana tabacum*) leaves & fruits and insect (*Tribolium castaneum).*

***Collection of Insects*:** The insects were collected from different godowns located in Layyah District.

***Rearing of Insects*:** The insect culture was maintained in jars placed in the incubator at 30±2°C and 60± 5% R.H to get the homogenous population. The culture medium was wheat and wheat flour sterilized at 60 °C for 60-90 minutes. Total 100 beetles from the heterogeneous population (20F+10M) were liberated in 250gm of wheat flour placed in different jars. The jars were covered with muslin cloth, tied with rubber bands to avoid the escape of beetles. Beetles were allowed to remain in the culture medium for 3 days for egg laying and then removed from jars with the help of sieves and fine camel hair brush for continuation of culture. The flour containing eggs was placed again in the same jars. This newly emerged culture was considered as homogeneous for the use of experimentation.

***Preparation of Plant Extracts*:** One kg fresh plant leaves of neem (*Azadirachta indica*); Kortuma (*Citrullus colocynthis*) and Tobacco (*Nicotiana tabacum*) were grinded after shade drying. Plant extracts of leaves were prepared using this method.

5 g of grind sample was taken in a flask and placed in Rotary Shaker at 320 rpm for 24 hours. After this, extracts were filtered with filter paper. From 100 % stock solution concentration was prepared.

BIOASSAY Mortality effect of plant extracts (Neem, Tobacco and Bitter apple) against *Tribolium castaneum*: What-man filter paper and Petri dishes were used for bioassay. Different concentration viz; 5%, 10% and 15 % were applied on the filter paper and let the filter paper to dry. Twenty-five adults of *Tribolium castaneum* was released in the Petri dish and then covered. Mortality rate of these adults against botanicals was recorded after 24, 48, 72 and 96 hours.

***Repellent effect of plants extracts on Tribolium castaneum*:** In this experiment, filter papers and petri dishes were used. Filter paper was cut into two halves. One half was treated with plant extract cane 5%, 10% and 15% respectively. While other half was treated with Acetone with the help of pipette. Cane viz. 5%, 10% and 15% of plant extracts were taken. The treated and untreated halves were attached to their opposite sites using adhesive tape and placed in Petri dishes. Twenty adults were released separately at the centre of each filter paper. The dishes then covered and placed in dark. Three replications were used for each concentration. Observations on the number of insects present on both treated and untreated halves were recorded after 24 hours in mild light.

Statistical Analysis

Statistical analysis of mortality data and repellency effects of botanicals against *Tribolium castaneum* was carried out. The effect of treatments was computed following CRD analysis of variance using software Statisticx 8.1.

RESULTS AND DISCUSSION

Table 1: Mortality effects of *Tribolium castaneum towards different neem concentrations*

Time	Neem Concentration			Mean
	5%	10%	15%	
24	33.33 D	51.667 C	38.88 D	49.29
48	67.500 B	65.00 B	65.00 B	81.83
72	68.750 B	69.417 B	69.250 B	93.14
96	78.333 A	76.667 A	76.667 A	109.22
Mean	61.99	65.71	62.49	

Table 2: Mortality effects of *T. castaneum towards different bitterapple concentrations*

Time	Bitter apple Concentration			Mean
	5%	10%	15%	
24	40.00 E	45.333 D	31.667 F	47.00
48	53.500 C	53.443 C	55.000 C	69.98
72	66.267 B	66.800 B	66.267 B	90.44
96	69.833 AB	72.250 A	73.000 A	103.69
Mean	57.41	59.48	56.52	

Table 3: Mortality effects of *T. castaneum towards different tobacco concentrations*

Time	Tobacco Concentration			Mean
	5%	10%	15%	
24	54.443 D	56.167 D	54.167 D	62.93
48	68.500 C	66.317 C	67.600 C	83.47
72	77.917 B	78.917 AB	79.417 AB	102.75
96	81.717 AB	83.333 A	82.100 AB	114.38
Mean	70.66	71.21	70.86	

In Table 1, mortaliliy effects were observed at different concentrations of neem viz 5%, 10% and 15% after different intarvals. After 24 hours intervals, 33 %, 51 % and 38 % was observed which is significant. These percentages were recorded according to parameters 5%, 10% and 15% concentrations. The maximum mortality percentage after 24 hours was at 10% concentration of neem solution. According to the time interval of 48 hour, 67 %, 65 %, 65 % of mortality of *Tribolium castaneum* was observed after 5%, 10% and 15% respectively. These digits shows more significancy than 24 hour results as among these, 5% concentration of neem after 48 hours showed more significant results than other concentrations. Similarly, 68 %, 69 % and 69 % mortality percentatges were observed after 72 hours of data. The almost similarity between results of 48 and 72 hour showed that there is less significancy and and little bit difference. While after 96 hours, maximum mortality of *Tribolium castaneum* was observed at 5% concentration as 78 % ,76 % and 76 % at 5%, 10% and 15% respectively. These mortality results revealed that while using neem solution against *Tribolium castaneum,* 5% concentration gave more effective and significant results than other concentrations.

In Table 2 bitterapple solution was used against *Tribolium castaneum* at different concentrations viz 5%,10% and 15% with the results 40,45 and 31 respectively. These are not much satisfactory results specially after 24 hours later. While comparing other results they are although significant but having a little bit variation among mortalities which varies from 40 to 73 at different interval (24-96 hrs.). In comparing with neem, Neem showed better results than bitter apple as more mortality was observed in neem as compared to Bitter apple.

In Table 3 tobacco extracts and then its solution used to check mortality effects of botanical against *Tribolium castaneum.* After 24 hours, it gave 54, 56, and 54 at different concentrations viz 5%,10% and 15% . The starting results of tobacco were more efficient and effective than other two botanicals. A great variation of mortality was observed atfter 48 hours (68 %, 66 %, 67 %), after 72 hours (77 %, 78 %, 79 %) and after 96 hours (80 %, 83 %, 82 %). So among all these botanicals and their application, tobacco showed more results than other botanicals at average after 24 and 48 hours and maximum after 72 and 96 hours.

Repellency Experiment

Table 4: Repellency effects of *T. castaneum towards different Neem concentrations*

Time	Neem Concentration			Mean
	5%	10%	15%	
24	13.333 C	17.333 B	22.333 A	25.67

Table 5: Repellency effects of *T. castaneum towards different Bitterapple concentrations*

Time	Bitterapple Concentration			Mean
	5%	10%	15%	
24	13.667 C	18.667 B	23.333 A	26.56

Table 6: Repellency effects of *T. castaneum towards different tobacco concentrations*

Time	Tobacco Concentration						Mean
	5%		10%		15%		
24	12.000	C	15.667	B	20.000	A	23.89

While calculating repellency, we have used different concentration of botanicals (Neem, Bitter apple and Tobacco) at only one-time interval (24 hrs.). In accordance with Table 4, repellency effect was 13 %, 17 % and 22 % at 5%, 10% and 15% respectively. This shows a good significant repellency percentage. The maximum repellency was at 15% concentration of Neem. In Table 5, repellency effect of bitter apple against *Tribolium castaneum* was as 13 %, 18 % and 23 % which is more than neem repellency effect. While in Table 6, data showed that maximum repellency at 15%. In comparison among all these, although all botanical showed good repellent effects, the best repellent was bitter apple, then neem and then tobacco.

CONCLUSION

It is concluded that all concentrations of each botanical were found to be effective as repellent against *Tribolium castaneum.* The repellency of bitter apple was better as compared to other repellents. The pest mortality rate was maximum at 10% concentration after 24 and 48 hours of time interval. The effectiveness of Neem and Kortumba were also found to be significant. The toxicity of Neem was lesser than tobacco. Moreover, Neem showed more repellent effect than only toxicity. These results suggest that the plant extracts evaluated in this study may be useful in repellent and toxicant formulations against *T. castaneum*.

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